

AMENDMENT

Amendments to the Claims:

Please amend the claims as follows, without prejudice:

In the Claims:

1. (Currently Amended) A method for amplifying one or more nucleic acids onto a bead comprising the steps of:
  - (a) forming a water-in-oil emulsion to create a plurality of aqueous microreactors wherein at least one of the microreactors comprises a-one single stranded nucleic acid template, a single bead with a first population comprising a plurality of molecules of a first primer species disposed thereon, ~~wherein the first primer species is capable of binding to the nucleic acid template, and an amplification reaction solution comprising a second population comprising a plurality of molecules of the first primer species and a plurality of molecules of a second primer species and reagents necessary to perform nucleic acid amplification, wherein the first primer species is capable of binding to the single stranded nucleic acid template, the second primer species is capable of binding to a complementary strand of the single stranded nucleic acid template, and a concentration of the second primer species that is substantially greater than that of the second population of the first primer species in the amplification reaction solution;~~  
~~(b) asymmetrically amplifying the single stranded nucleic acid template and the complementary strand to the template strand in the microreactors amplification reaction solution using the first and second primer species to form a population of amplified copies of the single stranded a complementary template nucleic acid, wherein substantially all of the molecules of the second population of the first primer species in the amplification reaction solution are depleted; and~~  
~~(c) capturing said binding a plurality of the asymmetrically amplified copies of the single stranded template nucleic acid to the first population of the first primer species on the bead in the microreactor using the first primer species, wherein a bead bound complementary strand is extended from the first primer species;~~

(d) breaking the aqueous microreactors to release at least one of the nucleic acid bound beads and the amplification reaction solution comprising unbound amplification products; and

(e) recovering the nucleic acid bound beads.

2. (Original) The method of claim 1, wherein a majority of the microreactors include a single nucleic acid.
3. (Previously Presented) The method of claim 1, wherein said amplification reaction solution is a polymerase chain reaction solution further comprising nucleotide triphosphates, a thermostable polymerase, and a buffer compatible with polymerase chain reaction conditions.
4. (Canceled).
5. (Canceled).
6. (Original) The method of claim 1, wherein said emulsion additionally contains emulsion stabilizers.
7. (Original) The method of claim 6, wherein said emulsion stabilizers are selected from the group consisting of Atlox 4912, Span 80, and combinations and mixtures thereof.
8. (Original) The method of claim 1 wherein said emulsion is heat stable.
9. (Original) The method of claim 8 wherein said emulsion is heat stable to 95°C.
10. (Original) The method of claim 1, wherein amplification is carried out by a method selected from the group consisting of transcription-based amplification, rapid amplification of cDNA ends, continuous flow amplification, and rolling circle amplification.
11. (Original) The method of claim 1, wherein the emulsion is formed by the dropwise addition of the nucleic acid templates, beads, and amplification reaction solution into an oil.
12. (Original) The method of claim 1, performed with at least 10,000 nucleic acids.
13. (Original) The method of claim 1, performed with at least 50,000 nucleic acids.
14. (Original) The method of claim 1, wherein the microreactors have an average size of 50 to 250 µm in diameter.

15. (Currently Amended) The method of claim 1, wherein ~~after step (e)~~ each bead captures binds more than 10,000 asymmetrically amplified amplification copies of ~~a~~the single stranded nucleic acid template.
16. (Withdrawn) A library comprising a plurality of nucleic acid molecules, wherein each nucleic acid molecule is separately immobilized to a different bead, and wherein each bead comprises over 1,000,000 clonal amplification copies of each nucleic acid molecule, wherein the library is contained in a single vessel.
17. (Withdrawn) The library of claim 16, wherein the nucleic acid molecules are selected from the group consisting of genomic DNA, cDNA, episomal DNA, BAC DNA, and YAC DNA.
18. (Withdrawn) The library of claim 16, wherein the genomic DNA is selected from the group consisting of animal, plant, viral, bacterial, and fungal genomic DNA.
19. (Withdrawn) The library of claim 18, wherein the genomic DNA is human genomic DNA or human cDNA.
20. (Withdrawn) The library of claim 16, wherein the bead has a diameter of 2 microns to 100 microns.
21. (Withdrawn) The library of claim 16, wherein the bead is a sepharose bead.
22. (Currently Amended) A method for amplifying a nucleic acid comprising the steps of:
  - (a) providing a one single stranded nucleic acid template to be amplified;
  - (b) providing a solid support material comprising a generally spherical bead having a diameter about 10 to about 80  $\mu\text{m}$ , wherein the bead comprises a plurality of molecules of a first population of a first primer species disposed thereon capable of binding to the nucleic acid template;
  - (c) mixing the nucleic acid template and the bead in an amplification reaction solution comprising a plurality of molecules of a second population of the first primer species, a second primer species and reagents necessary to perform a nucleic acid amplification reaction in a water-in-oil emulsion, wherein the first primer species is capable of binding to the single stranded nucleic acid template, the second primer species is capable of binding to a complementary strand

of the single stranded nucleic acid template and a concentration of the second primer species is substantially greater than the second population of the first primer species in the amplification reaction solution;

- (d) asymmetrically amplifying the single stranded nucleic acid template and the complementary strand of the single stranded nucleic acid template in the amplification reaction solution using the second population of the first primer species and the second primer species to form a population of amplified copies of a complementary the single stranded template nucleic acid, wherein substantially all of the molecules of the second population of the first primer species in the reaction solution are depleted; and
- (e) capturing said copies binding a plurality of the asymmetrically amplified copies of the single stranded template nucleic acid to the first population of the first primer species on the bead using the first primer species, wherein a bead bound complementary strand is extended from the first primer species; and
- (f) recovering the nucleic acid bound beads.

23. (Withdrawn) A kit for conducting nucleic acid amplification of a nucleic acid template comprising:

- (a) a nucleic acid capture bead;
- (b) an emulsion oil;
- (c) one or more emulsion stabilizers;
- (d) instructions for performing the method of claim 1 or claim 22.

24. (Original) The method of claim 1 or claim 22 further comprising the step of enriching for beads which bind amplified copies of the nucleic acid away from beads to which no nucleic acid is bound, the enrichment step selected from the group consisting of affinity purification, electrophoresis and cell sorting.

25. (Original) The method of claim 24 wherein the enrichment step is performed by affinity purification with magnetic beads that bind nucleic acid.

26. (Original) The method of claim 1 or 22, wherein at least 100,000 copies of each target nucleic acid molecule are bound to each bead.
27. (Original) The method of claim 1 or 22, wherein at least 1,000,000 copies of each target nucleic acid molecule are bound to each bead.
28. (Original) The method of claim 1 or 22, wherein between at least 1 to 20,000,000 copies of each target nucleic acid molecule are bound to each bead.
29. (Original) The method of claim 1 or 22, wherein the beads are sepharose beads.
30. (Canceled).
31. (Canceled).
32. (Original) The method of claim 25, further comprising the steps of: separating the template carrying beads and magnetic bead; and removing the magnetic beads with a magnetic field.
33. (Original) The method of claim 32, wherein the separating is achieved by incubation at a temperature greater than 45°C or by incubating the template carrying beads and the magnetic beads in a solution with a basic pH.
34. (Currently Amended) A method for producing a clonal population of nucleic acids, comprising:
  - (a) providing a plurality of single stranded nucleic acid templates from 50-800 bp in length and beads each comprising a first population of a plurality of molecules of a first primer species disposed thereon capable of binding to the nucleic acid templates;
  - (b) mixing the single stranded nucleic acid templates and the beads in an biological amplification reaction solution that comprises a second population of a plurality of molecules of the first primer species, a plurality of molecules of a second primer species and reagents necessary to amplify the nucleic acid templates, wherein and a concentration of the second primer species that is substantially greater than the second population of the first primer species in the amplification reaction solution, wherein the first primer species is capable of binding to the

single stranded nucleic acid template and the second primer species is capable of binding to a complementary strand of the single stranded nucleic acid template;

(c) forming an emulsion to create a plurality of microreactors comprising the nucleic acid templates, beads, and the biological amplification reaction solution, wherein at least one of the microreactors comprises a single nucleic acid template and a single bead encapsulated in the biological amplification reaction solution, wherein the microreactors are contained in the same vessel.

35. (Withdrawn) The method of claim 34 further comprising transcribing and translated the nucleic acids to generate at least 10,000 copies of an expression product.

36. (Withdrawn) The method of claim 35, wherein said expression product is bound to said beads by a binding pair selected from the group consisting of antigen/antibody, ligand/receptor, 6Xhis/nickel-nitrilotriacetic acid, and FLAG tag/FLAG antibody binding pairs.

37. (Withdrawn) The method of claim 35, wherein the method produces a clonal population of proteins.

38. (Withdrawn) The method of claim 37, wherein the proteins are selected from the group consisting of antibodies, antibodies fragments, and engineered antibodies.

39. (Withdrawn) An emulsion comprising a plurality of thermostable microreactors, wherein the microreactors are 50 to 200  $\mu\text{m}$  in diameter and comprise a biological reaction solution..

40. (Withdrawn) The emulsion of claim 39, wherein the biological reaction solution comprises reagents for performing polymerase chain reaction amplification.

41. (Withdrawn) The emulsion of claim 39, wherein the biological reaction solution comprises reagents for performing coupled transcription and translation reactions.

42. (Withdrawn) The emulsion of claim 40 or claim 41, wherein the plurality of microreactors comprise a nucleic acid template.

43. (Withdrawn) The emulsion of claim 42, wherein the plurality of microreactors comprise one or fewer nucleic acid templates.

44. (Withdrawn) The emulsion of claim 43, wherein the plurality of microreactors comprise one or fewer beads that bind to the nucleic acid templates.